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ABSTRACT: The morphology of late fourth and early fifth stages of *Ascaris suum* in swine was studied with light and scanning electron microscopy. Late fourth-stage larvae have dome-shaped lips, that are broadest at the base and incompletely separated laterally. The lips bear coarse, triangular denticles, irregularly spaced on the internomedial and internolateral walls. Large double papillae are located on the middle of the external lip surface. The cervical region gradually increases in width posteriorly. The late fourth-stage cuticle is marked by a coarse transverse striation and bears incomplete longitudinal ridges, creating a brickwork pattern; longitudinal alae are present. Late fourth and early fifth stages have overlapping body lengths; late fourth-stage larvae at 21–24 DAI range from 13 to 27 mm long, and early fifth stages at 23–24 DAI range from 22 to 36 mm long. Early fifth-stage lips are truncate, broadest at the level of the prominent double papillae, constricted at the base and widely separated laterally. The lips bear a single row of fine, regularly spaced denticles on the internal margins. The prominent double papillae are located slightly anterior to the middle of the external lip surface. The cervical region sharply increases in width giving a shouldered appearance. The cuticle of early fifth stages is finely striated without markings; longitudinal alae are absent.

Reliable anatomical characters and morphometrics for the late developmental stages of *Ascaris suum* are still lacking although the species has been studied by many investigators. Douvres et al. (1969) described the life cycle and the early development of *A. suum* in swine from the ingested infective egg through early fourth-stage larvae. The present report describes the structure of late fourth and early fifth stages of *A. suum* in swine.

Materials and Methods

Purebred Hampshire swine from the closed breeding herd maintained at this institute were inoculated with a single oral dose of 500 *A. suum* eggs. The dose level of 500 eggs was selected for reasons explained by Tromba (1978). The swine had been farrowed and maintained under conditions precluding previous exposure to *A. suum*. Procedures for preparation of inocula and necropsy were those reported by Douvres et al. (1969) and Madden and Tromba (1976). *Ascaris suum* in late fourth stage (includes larvae closely enveloped in a sheath), fourth ecdysis, and early fifth stage were recovered from washings of the small intestine. The nematodes were killed and fixed in Bles' fixative (Meyer and Olsen, 1971), cleared for study in phenol-alcohol, and studied in temporary wet mounts. Photomicrographs were taken with a 35-mm camera, mounted on a microscope equipped with an interference contrast attachment. Scanning electron micrographs were prepared according to the methods of Madden and Tromba (1976). Morphological terminology follows Chitwood and Chitwood (1950). Nematodes have been deposited in the U.S. National Parasite Collection as USDA Parasite Collection Nos. 68501, 68518, and 68519 for specimens collected 21, 23, and 24 days after inoculation (DAI), respectively.

Table 1. Comparison of characters useful in differentiating late fourth and early fifth stages of *Ascaris suum*.

Characters	Late fourth stages	Early fifth stages
Lips	Dome-shaped Broadest at base Incompletely separated laterally External surface rounded	Truncate Broadest at level of double papillae, lip base narrow Widely separated laterally External surface flat
Denticles	Coarse, appear triangular Irregularly spaced	Fine, may appear blunt or triangular Regularly and evenly spaced
Cervical region	Body gradually increasing in width posterior to lips	Body sharply increasing in width behind lips giving a shouldered appearance
Cuticle	Annules wide Longitudinal ridges present on annules Longitudinal alae present	Annules narrow Longitudinal ridges absent Longitudinal alae absent

Results

Nematodes were recovered from swine killed 21, 23, and 24 DAI; the pig killed 22 DAI was negative for nematodes. At 21 DAI all 14 recovered larvae were in the late fourth stage. At 23 DAI 84 of 87 recovered nematodes were in the late fourth stage; three were in the early fifth stage. At 24 DAI five of 26 recovered nematodes were late fourth-stage larvae, one was in fourth ecdysis, and 20 were in the early fifth stage. The most useful characters for differentiating late fourth and early fifth stages of *Ascaris suum* are the labial, cervical, and cuticular structures (Table 1). Ranges of measurements of late fourth stage, fourth ecdysis and early fifth stages of *A. suum* are given in Table 2.

Late fourth-stage larvae (Figs. 1–3, 7–9, 13, 14)

Three dome-shaped lips separated internomedially, internolaterally, and incompletely separated laterally (Figs. 1, 2). Dorsal lip slightly broader than subventral lips; all lips broadest at lip base; and each lip with longitudinal groove on internomedial wall. External surface of lips rounded in overall appearance (Fig. 7). Denticles on inner lip margins coarse, triangular, and irregularly spaced in single row (Fig. 2, insert). Dorsal and subventral lips bearing large double papillae of external circle in middle of externolateral lip surface (Figs. 1, 2, 8). Each subventral lip bearing small externolateral papilla (also of external circle) slightly anterior to double papilla; amphid adjacent to externolateral papilla (Fig. 2). Cervical region gradually increasing in width posterior to lips (Fig. 7). Interlabia absent. Cuticle coarsely transversely striated, annules 0.017–0.029 mm wide at base of esophagus; incomplete longitudinal ridges present on annules (Figs. 3, 9); longitudinal alae present extending from base of lips almost to tail tip.

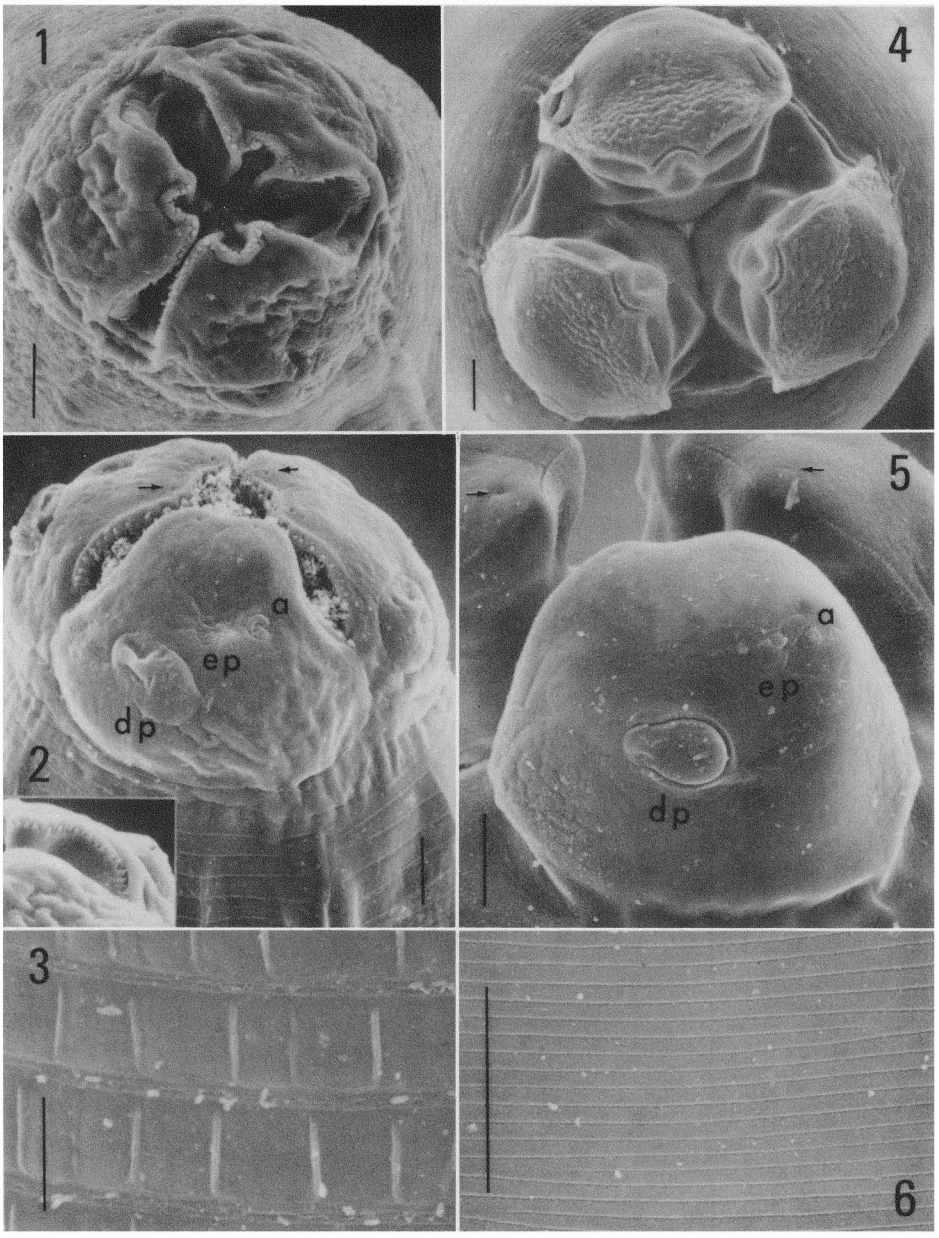
Fourth ecdysis

Single female recovered. Body emerged from fourth-stage cuticle to level of vulva. Lips characteristic of fifth stage; cuticle of anterior half of body finely striated. Posterior part of body enclosed within fourth-stage cuticle.

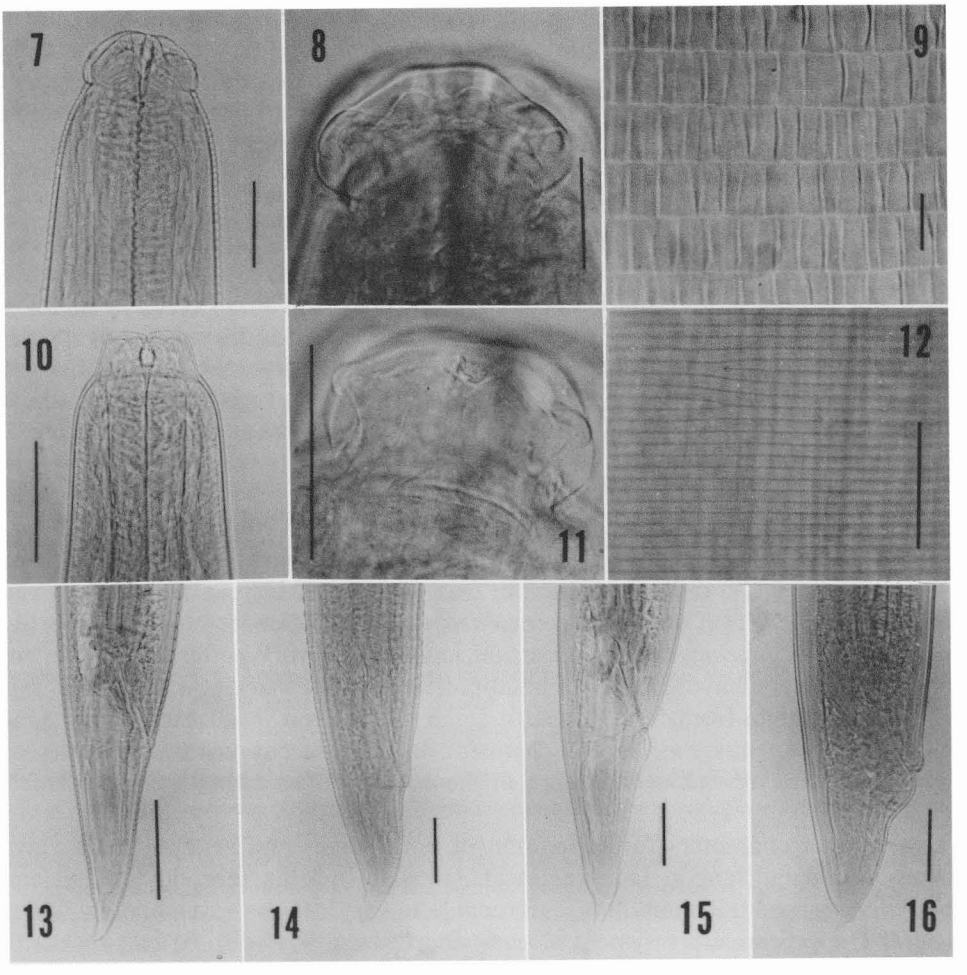
Table 2. Body measurements of late fourth stage, fourth ecdysis, and early fifth stages of *Ascaris suum* collected from experimental infections in swine.*

	Late fourth stages 21–24 DAI		Fourth ecdysis 24 DAI	Early fifth stages 23–24 DAI	
Anatomical feature	Males (19)	Females (13)	Female (1)	Males (11)	Females (10)
Total length	15.30–24.12 (18.99)	13.38–27.00 (20.33)	27.18†	21.96–35.64 (26.43)	24.30–34.92 (28.60)
Width (midbody)	0.31–0.51 (0.40)	0.32–0.53 (0.45)	0.51	0.41–0.56 (0.48)	0.50–0.62 (0.54)
Esophagus‡	1.32–1.88 (1.65)	1.41–2.01 (1.71)	2.03	1.18–2.35 (2.00)	1.94–2.39 (2.10)
Excretory pore‡	0.35–0.46 (0.42)	0.40–0.48 (0.43)	0.38	0.38–0.50 (0.42)	0.40–0.48 (0.43)
Spicule length	0.11–0.25 (0.18)	—	—	0.19–0.34 (0.24)	—
Vulva‡	—	9.02–14.63 (11.42)	15.22	—	12.91–18.01 (15.07)
Tail length	0.17–0.24 (0.21)	0.27–0.35 (0.31)	0.36	0.20–0.30 (0.24)	0.33–0.44 (0.39)
Width of annule§	0.017–0.029 (0.022)	0.021–0.029 (0.024)	0.002	0.002–0.003 (0.002)	0.002–0.003 (0.002)

* Ranges (and averages) in millimeters.
† Length of female in fourth ecdysis does not include the sheath.
‡ Structures were measured from the anterior end.
§ Annule was measured in area at base of esophagus.



Figures 1–6. *Ascaris suum*, **scanning electron micrographs of the labial and cuticular structure of late fourth and early fifth stages. Scale bars 25 μm. Figures 1–3. Late fourth stages. 1. En face view, showing lip denticles and incomplete lateral lip separation. 2. Dome-shaped subventral lip, showing large double papilla (dp), externolateral papilla (ep), amphid (a), and the internal circle of cephalic papillae (at arrows). Insert: Enlargement of dorsal lip, showing triangular shape of denticles in lateral view. 3. Cuticle (just anterior to excretory pore), showing the brickwork pattern of incomplete longitudinal ridges. Figures 4–6. Early fifth stages. 4. En face view, showing wide lateral lip separation. 5. Truncate subventral lip, showing prominent double papilla (dp), externolateral papilla (ep), amphid (a), and the internal circle of cephalic papillae (at arrows). 6. Cuticle (posterior to lips), showing fine transverse striae and annules without markings.**



Figures 7–16. ***Ascaris suum*, interference-contrast light micrographs of the labial and cuticular structure of late fourth and early fifth stages. Scale bars 100 μm in Figures 7–8, 10–11, 13–16; scale bar 25 μm in Figures 9, 12. 7. Late fourth-stage anterior extremity, dorsal view, showing gradual increase in width from lip base. 8. Dome-shaped dorsal lip of late fourth-stage larva, showing two large double papillae. 9. Coarsely striated cuticle of late fourth-stage larva (near base of esophagus), showing incomplete longitudinal ridges. 10. Early fifth-stage anterior extremity, dorsal view, showing shouldered appearance from narrowed lip base. 11. Truncate dorsal lip of early fifth stage, showing two prominent double papillae. 12. Finely striated cuticle of early fifth stage (near base of esophagus). 13. Late fourth-stage female tail. 14. Late fourth-stage male tail. 15. Early fifth-stage female tail. 16. Early fifth-stage male tail.**

Early fifth stages (adults) (Figs. 4–6, 10–12, 15, 16)

Lips truncate, broader than tall, separated internomedially, internolaterally, and widely separated laterally (Figs. 4, 5). All lips broadest at level of prominent double papillae; narrowed at base. External surfaces of lips relatively flat in overall view (Fig. 10). Denticles on internal lip margins fine, triangular to blunt, and regularly and evenly spaced in single row (Fig. 5). Dorsal and subventral lips bearing prominent double papillae of external circle slightly anterior to middle of

lip surface (Figs. 4, 5, 11). Subventral lips each bear an externolateral papilla of external circle anterior to large double papilla and porelike amphid next to externolateral papilla (Fig. 5). Cervical region sharply increases in width at base of lips giving shouldered appearance (Fig. 10). Cuticle very finely striated, annules 0.002–0.003 mm wide at base of esophagus; annules without ridges (Figs. 6, 12); longitudinal alae absent.

Discussion

The shape of the lips, denticular structure, shape of the cervical region, and cuticular structure differ markedly between the stages (Table 1). The denticles in fourth-stage lips can be seen by focusing through the lip surface to the denticular ridge, however, denticles in fifth-stage lips are not easily seen by light microscopy. Madden and Tromba (1976) found that denticle size is directly related to the size and age of the nematodes and that denticles appear triangular to blunt, depending upon the angle of view. The "brickwork" pattern of fourth-stage cuticle (Douvres et al., 1969) is not present on the anteriormost annules (Fig. 2) but begins slightly anterior to the excretory pore (Fig. 3) and continues to the tail tip. Fifth-stage cuticle is finely striated and the annules are narrow without markings. A similar change in cuticular structure was reported (Pilitt et al., 1979) between fourth and early fifth stages of *Parascaris equorum* (Goeze, 1782). In both *A. suum* and *P. equorum,* late fourth stages have a brickwork-patterned cuticle, but early fifth stages have a finely striated cuticle without markings. However, this change in cuticle from fourth to fifth stage is not seen in all ascaridoids. One example, the genus *Sulcascaris* (parasitic in molluscs as larvae, in turtles as adults), retains a brickwork pattern in the cuticle of the fifth stage (Lichtenfels et al., 1978).

Additional characters for separation of both stages and sexes include vulva location and tail length. Douvres et al. (1969), using the reproductive system, body length, and tail characters were unable to separate the sexes in early fourth stage. The reproductive system of early fourth stages is still limited to a multicellular genital primordium. In late fourth-stage females the prepatent vulva is located slightly posterior to midbody, beneath the fourth-stage cuticle. The vagina separates into two posteriorly directed uteri just posterior to the vulva. The patent vulva of the early fifth-stage female is located about midbody. The female reproductive tract is easier to locate in the fourth stage than in the early fifth stage. Late fourth-stage female tails (Fig. 13) are longer than fourth-stage male tails (Fig. 14). Genital papillae and developing spicules can be seen in males under the fourth-stage cuticle. In some fourth-stage males the weakly sclerotized spicules may become overcleared in phenol-alcohol. Tails of early fifth-stage females (Fig. 15) are longer than tails of fifth-stage males (Fig. 16). Males have two equal spicules and genital papillae; caudal alae are absent.

All nematodes described in this study were from a single series of swine inoculated simultaneously. The fourth ecdysis occurred between 22 and 25 DAI in these infections. In other experimental inoculations of swine at this institute, the fourth ecdysis of *A. suum* has been observed at 23 DAI. Roberts (1934) proposed that the fourth ecdysis occurred in the small intestine of swine between 21 and 29 DAI; two of his 29 DAI larvae were in fourth ecdysis (17.5 mm and 22.5 mm long, respectively). Schwartz (1959) suggested that the natural elimination of

ascarid larvae from swine between 21 and 30 days corresponded to the beginning of the fourth molt.

The body lengths of late developing stages overlap and if used alone are unreliable for separation of stages (Table 2). Roberts (1934) relied on body lengths to identify larval ascarids. He also used anatomical characters to separate stages of development, but frequently the characters he used were common to more than one larval stage. Douvres et al. (1969), in their description of early and middle developmental phases of *A. suum* in swine, also reported that body lengths often overlapped between stages; however, they presented additional characters of head, lip, and other body features for the separation of the different phases and stages of development. The most useful characters presented here for differentiating late fourth and early fifth stages are labial and cuticular structure (summarized in Table 1).

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